 DOI: 10.35382/TVUJS.15.1.2025.117

OPTIMIZATION OF RED PUMPKIN (*Cucurbita moschata*) SACCHARIFICATION USING LINEAR CONVOLUTION FOR MULTI-OBJECTIVE FUNCTIONS

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Abstract – *This study investigates optimizing the saccharification process to obtain desired levels of rapidly digestible starch (Y1), slowly digestible starch (Y2), and resistant starch (Y3) in red pumpkin (Cucurbita moschata). Saccharification involves treating liquefied starch with glucoamylase to convert it into glucose. The experiment examines the influence of glucoamylase concentration, temperature, and hydrolysis time on these starch fractions. A central composite design with a multi-objective function was employed to create a multivariate model. The linear convolution method, along with the convolution function gradient climbing method, was used to analyze the multi-objective data and identify optimal solutions based on experimental results. The established linear convolution function equation demonstrated a high degree of accuracy (R^2 = 0.9846, CV = 0.71%) and statistical significance ($p < 0.0001$). The chosen convolution function ($Y_{convmax}$ = 301.312 mg/g) yielded values close to the desired ranges for $Y1_{min}$, $Y2_{max}$, and $Y3_{max}$. These values translated to Y1 = 489.390 mg/g, Y2 = 151.236 mg/g, and Y3 = 357.348 mg/g under the corresponding experimental conditions: glucoamylase concentration (X1) of 119.384 U/ml, temperature (X2) of 57.051^oC, and hydrolysis time (X3) of 179.890 minutes.*

Keywords: glucoamylase, pumpkin starch, rapidly digestible starch, reducing sugar, slowly digestible starch.

I. INTRODUCTION

Red pumpkin (*Cucurbita moschata*) is a valuable source of starch, a complex carbohydrate that plays a vital role in human nutrition. However, not all starches are created equal. Starch can be classified into three main types based on its digestion rate: rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS) [1]. This classification has significant health implications. RDS is quickly broken down by the body, leading to blood sugar spikes [2]. In contrast, SDS offers sustained energy and may promote gut health. SDS offers a distinct advantage over rapidly digestible starches. It provides a gradual and sustained release of glucose into the bloodstream, potentially aiding in the management of diabetes, obesity, and cardiovascular diseases [3–5]. Even more intriguing RS, which remains undigested in the small intestine and undergoes fermentation in the colon. This process has been linked to improved blood sugar control, modulation of gut health, and potential benefits for weight management [6–9]. Notably, studies have consistently shown that consuming RS reduces blood sugar spikes compared to other carbohydrates, leading to an approved health claim by the European Union [10]. Due to their health-promoting properties, both RS and SDS are gaining recognition as 'nutraceutical starches' [11]. While further research is needed to understand the effects of different RS types fully, the current evidence suggests that incorporating more RS and SDS into our diets could offer significant health advantages.

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Received date: 2 January 2025; Revised date: 10 March 2025; Accepted date: 25 March 2025

The saccharification process can be optimized to manipulate the proportions of these starch fractions in red pumpkin. This process uses enzymes to convert starch into simpler sugars. The efficiency of this conversion directly affects the final amounts of RDS, SDS, and RS. This study employs a novel approach using the linear convolution method for multi-objective functions to optimize the saccharification process. Through the process, this study aims to identify conditions that achieve the desired levels of each starch fraction, ultimately enhancing the nutritional value of red pumpkin.

II. LITERATURE REVIEW

Recent studies have focused on increasing the content of RS in various foods to enhance nutritional value and human health [12, 13]. Various approaches have been employed, such as study of Ho Thi Hao et al. [12] successfully increased the RS3 content in jackfruit seed starch to 82% through a heat-moisture treatment process. Optimal conditions were achieved with a sample moisture content of 21%, a temperature of 111^oC, and an incubation time of 12 hours. Meanwhile, Gurunathan et al. [13] developed a rice mutant (γ278) with higher RS content using gamma irradiation. The study identified gene mutations associated with increased RS, particularly in the GBSSI, SSIIa, and SSIIIa genes. This mutant rice variety exhibited an amylose content of 26.18% and an RS content of 8.68%.

Several studies have explored the use of enzymes to enhance RS content in various starches [14–18]. For instance, Nguyen Thi Mai Huong et al. [14] investigated the enrichment of RS in mung bean starch using pullulanase. Optimal conditions were found at 17.5 hours, with a pullulanase concentration of 45 U/g and a starch-to-water ratio of 1:20, resulting in a maximum RS content of 60.98%. Similarly, Zhang et al. [15] employed pullulanase to hydrolyze corn starch and produce RS, achieving a 44.7% RS content under specific conditions. Pongjanta et al. [16] utilized pullulanase to produce RS type III from rice starch, obtaining a 19.81% RS content with a pullulanase concentration of 12 U/g. Furthermore, Le Thi Bich Phuong et al. [17] optimized the saccharification process of waxy corn starch using glucoamylase. Response surface methodology analysis revealed that the optimal conditions for glucoamylase hydrolysis of corn starch were a 0.12% enzyme concentration, a temperature of 66.76^oC, and a reaction time of 237 to 240 minutes, resulting in the highest reducing sugar content of $13.61\pm0.143\%$. Shin et al. [18] studied the production of SDS from sorghum starch using isoamylase, finding that an 8-hour hydrolysis time followed by storage at 1^oC for three days yielded the highest SDS content of 27.0%.

Shen et al. [7] demonstrated that RS content is influenced not only by processing methods but also by several factors, including the structure of starch granules, the amylose-amylopectin ratio, protein and lipid content, storage conditions, and processing parameters. Zhang et al. [19] highlighted that proteins can form a protective layer around starch granules, thereby limiting the accessibility of hydrolytic enzymes. This protective layer hinders the interaction between enzymes and starch, ultimately reducing the saccharification rate. Similarly, Parada et al. [20] and Hu et al. [21] suggested that lipids can also create a barrier around starch granules, particularly at high concentrations. The lipid layer obstructs enzyme access, as lipid molecules bind tightly to starch, forming lipid-starch bridges that resist enzymatic degradation. As a result, the saccharification efficiency is reduced. Doan Thu Huong et al. [22] further confirmed the wide range of RS content, from 1% to 42%, depending on the source, structure, and processing methods. These studies highlight the feasibility of enhancing RS content in foods. However, further research is needed to explore the combination of different methods and evaluate the interactions between various influencing factors aiming to optimize this process.

III. MATERIALS AND METHODS

A. Materials

Yellow-orange-skinned pumpkins, with a diameter of 10–15 cm, a flesh thickness of approximately 3.5 cm, and a weight of 2.2–2.5 kg, were purchased from Ba Thu agent at Tra Vinh market. The thermostable α-amylase enzyme, sourced from *Bacillus licheniformis* and supplied by Novozyme, has a published activity of 120 KNU-S/g. It is a liquid form with a viscosity ranging from 1 to 25 cPs, an optimal pH range of 5.5–6.5, and a temperature optimum between 85–90^{o}C. Glucoamylase, obtained from Oxford Lab in India (purchased through Phuongtram Agricultural Product & Chemical Trading Co., Ltd., Vietnam), is a brown liquid enzyme with a published activity of 300 U/ml. It has a pH range of 3.0–5.5, with an optimal temperature range of 60–65^{o}C, and is sourced from *Aspergillus sp*. The chemicals used include 3,5-dinitrosalicylic acid (DNS), KOH, CH_3COOH, CH_3COONa, NaOH, D-glucose, and $KNaC_4H_4O_6.4H_2O$, all sourced from China.

B. Methods

(1) Sample preparation: Raw red pumpkin was peeled, washed, and ground with water in a 1:2 ratio. The mixture was then diluted with water (1:4) to reduce viscosity and filtered. The filtrate was air-dried at room temperature for 12 hours, followed by further drying at 50^{o}C until the moisture content reached 10–12%. The dried sample was then finely ground using a sieve with a mesh size of $\geq$ 35, resulting in red pumpkin starch. The starch samples were then analyzed for moisture by drying to constant weight; ash content was determined by complete combustion at high temperature (500–600^{o}C); total lipid content was determined by the Soxhlet method; protein content was determined by the micro-kjendahl method; and amylose content was determined by the iodine method.

(2) Enzymatic hydrolysis: Red pumpkin starch was mixed with water in a 1:4 ratio and gelatinized at 85^{o}C for 15 minutes, following the protocol established by Zhang et al. [23]. α-Amylase was then added to the gelatinized mixture for enzymatic hydrolysis, following the approach described by Hoang et al. [24]. After hydrolysis, the mixture was cooled down in preparation for the subsequent saccharification step.

(3) Saccharification optimization: A central composite design with three factors (glucoamylase concentration, hydrolysis temperature, and hydrolysis time) was employed to optimize the saccharification process for red pumpkin starch. The encoded data for these independent variables is presented in Table 1. The dependent responses are RDS ($Y1_{min}$), SDS ($Y2_{max}$), and RS ($Y3_{max}$). The proposed polynomial regression equation is applied as Equation (1).

$$Y = \beta_0 + \sum_{i=1}^{n} \beta_i X_i + \sum_{i=1}^{n} \beta_{ii} X_i^2 + \sum \sum_{i<j=1}^{n} \beta_{ij} X_i X_j + e \quad (1)$$

Where: Y is the dependent variable, β_o is the intercept coefficient; β_i is the coefficient of the quadratic equation; β_{ii} is the coefficient of the quadratic equation of the variable X_i; β_{ij} is the interaction coefficient; and e is the random error.

The fixing factor includes a sample amount of 50 g and a pH of 4.5.

The RDS, SDS, and RS content in the research samples were analyzed and determined based on the formula of Englyst et al. [1], presented in Equation (2).

$$\left.\begin{aligned} &TS = (TG - FG)*0.9 \\ &RDS = (G_{20} - FG)*0.9 \\ &SDS = (G_{120} - G_{20})*0.9 \\ &RS = TS - (RDS + SDS) \text{ or } RS = (TG - G_{120})*0.9 \end{aligned}\right\} \quad (2)$$

With: FG – glucose content at time 0 minutes; G20 – glucose content released after hydrolysis 20 minutes; G120 – glucose content released after hydrolysis 120 minutes; and TG – total glucose content.

C. Statistical analysis

The results were processed using statistical software such as SPSSv27 and Excel 2021. The optimization experiments were processed using Design-Expert 13.0.0.

Table 1: Data coding for saccharification experiments according to CCD

Coded symbols	Independent variable	Units	Levels				
			–1.5	–1	0	1	+1.5
X_1	Glucoamylase	U/ml	45	60	90	120	135
X_2	Temperature	°C	45	50	60	70	75
X_3	Time	minute	30	60	120	180	210

IV. RESULTS AND DISCUSSION

A. Red pumpkin starch characterization

The red pumpkin starch was isolated and characterized. The final moisture content of the dried starch (11.26%) was well within acceptable storage standards (< 13%) (Table 2). Starch, primarily composed of amylose and amylopectin, contains minor components like proteins and lipids. These minor components play a crucial role in the saccharification process and the properties of modified starch. The amylose/amylopectin ratio directly impacts saccharification efficiency [25].

Table 2: Characterization of pumpkin starch

Humidity (%)	Ash (%)	Lipid (%)	Protein (%)	Amylose (%)
11.26	0.21	3.32	10.65	24.88
±0.007	±0.011	±0.068	±0.10	±0.13

Note: all data are the means of duplicate experiments ± standard deviations

Amylose, with its linear chain structure, tends to coil to form stable structures that are more resistant to hydrolysis by enzymes (amylase) compared to branched amylopectin. Consequently, starches with higher amylose content may exhibit lower efficiency during saccharification [25].

Proteins can form a protective layer around starch granules, limiting the accessibility of hydrolytic enzymes [19]. This reduces the saccharification rate as enzymes struggle to interact effectively with the starch. However, several proteins, when broken down during saccharification, can aid the enzymatic hydrolysis process by acting as co-factors [19]. Lipids, especially at high concentrations, also create a barrier around starch granules [20]. This lipid layer impedes enzyme access, as lipid molecules bind tightly to starch, forming lipid-starch bridges that resist enzymatic breakdown [20, 21]. This leads to decreased saccharification efficiency. Analysis of red pumpkin starch revealed a moderate amylose content, while protein and lipid levels were relatively high. This suggests that these components are likely to reduce the efficiency of the saccharification process.

B. Effect of glucoamylase concentration, temperature, and hydrolysis time on RDS, SDS, and RS

An empirical matrix model was employed to generate the dependent variable values, as presented in Table 3.

Table 3: Experimental results

Run order	X_1 U/g	X_2 °C	X_3 minutes	Y_{conv} mg/g	Run order	X_1 U/g	X_2 °C	X_3 minutes	Y_{conv} mg/g
2	-1	-1	-1	269.52	15	1	1	1	293.66
11	1	-1	1	298.49	30	0	0	0	294.50
7	1	1	-1	255.14	29	0	0	0	292.96
9	-1	-1	1	261.96	21	0	-1.5	0	283.65
5	-1	1	-1	283.30	17	-1.5	0	0	274.68
16	1	1	1	293.80	13	-1	1	1	277.96
4	1	-1	-1	261.84	14	-1	1	1	277.46
1	-1	-1	-1	269.42	22	0	-1.5	0	283.71
19	1.5	0	0	285.06	25	0	0	-1.5	258.43
20	1.5	0	0	285.02	6	-1	1	-1	283.62
3	1	-1	-1	260.40	28	0	0	1.5	286.98
27	0	0	1.5	284.90	31	0	0	0	289.48
24	0	1.5	0	285.13	23	0	1.5	0	285.03
32	0	0	0	294.88	12	1	-1	1	301.12
8	1	1	-1	256.99	10	-1	-1	1	261.36
18	-1.5	0	0	274.78	26	0	0	-1.5	265.77

The experimental matrix table describes a set of data points (X1, X2, X3) that represent the influence of various factors on the response variables Y1, Y2, and Y3. Any changes in these data points will lead to different experimental outcomes, impacting the dependent response variables $Y1_{min}$, $Y2_{max}$, and $Y3_{max}$. However, achieving the ideal values ($Y1_{min}$, $Y2_{max}$, $Y3_{max}$) for all three responses simultaneously is not feasible.

Therefore, the goal is to identify a compromise solution where the observed responses (Y1, Y2, Y3) are as close as possible to the desired values ($Y1_{min}$, $Y2_{max}$, $Y3_{max}$). To achieve the values, a linear convolution method will be employed, as described by the general Equation (3).

$$Y_{conv} = \alpha_1 Y1 + \alpha_2 Y2 + \alpha_3 Y3 \quad (3)$$

Where: α_1, α_2, α_2 are the importance coefficients corresponding to the objective functions Y1, Y2, and Y3.

Prioritizing higher SDS and RS content over RDS (due to RS being a beneficial source of human starch), we assign higher importance coefficients (α) to Y2 and Y3. Consequently, α_1 = 0.2, α_2 = 0.4 and α_3 = 0.4 are chosen.

This selection yields the following linear convolution function Equation (4).

$$Y_{conv} = 0.2Y1 + 0.4Y2 + 0.4Y3 \quad (4)$$

The model's performance was evaluated by using several methods. First, the calculated Yconv values based on the experimental matrix were compared to the actual data (Table 3). Figure 1a shows that the residual data follows a normal distribution N(0,1). In Figure 1(b), the actual and predicted model values align well. Figure 1c demonstrates that the residual values and run numbers around the coordinate 0.00, indicating good predictive results. Overall, these analyses demonstrate that the data satisfies the required assumptions for the model.

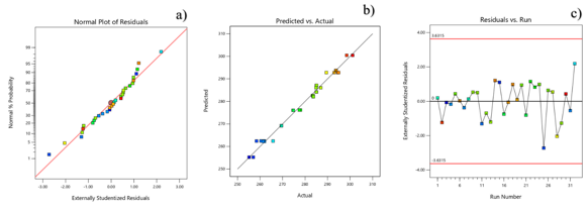

Fig. 1: Linear convolution function data distribution

Linear convolutional regression is presented in Equation (5).

$$Y_{conv} = 292.794 + 2.711X_1 + 1.681X_2 + 7.884X_3 - 5.147X_1X_2 + 11.238X_1X_3 - 5.642X_1^2 - 3.646X_2^2 - 8.249X_3^2 \quad (5)$$

Table 4: ANOVA analysis for Y_{conv} function results

Source	SS[a]	df[b]	MS[c]	F-value	*p*-value	
Model	5459.10	9	606.57	156.25	< 0.0001	Significant
X_1	183.75	1	183.75	47.33	< 0.0001	
X_2	70.62	1	70.62	18.19	0.0003	
X_3	1554.01	1	1554.01	400.31	< 0.0001	
X_1X_2	423.79	1	423.79	109.17	< 0.0001	
X_1X_3	2020.98	1	2020.98	520.60	< 0.0001	
X_2X_3	0.2673	1	0.2673	0.0689	0.7954	
X_1^2	472.00	1	472.00	121.59	< 0.0001	
X_2^2	197.13	1	197.13	50.78	< 0.0001	
X_3^2	1008.93	1	1008.93	259.90	< 0.0001	
Residual	85.40	22	3.88			
Lack of Fit	31.47	5	6.29	1.98	0.1328	Not significant
Pure Error	53.93	17	3.17			
R^2	0.9846					
Adjusted R^2	0.9783					
Adequate Precision	41.0174					

Note: [a] sum of squares, [b] degree of freedom, [c] mean of squares

An Analysis of Variance (ANOVA) was performed (Table 4). The results confirmed the model's statistical significance ($p < 0.0001$) and good fit to the data (Lack of fit $p > 0.05$). Model reliability (R^2 = 0.9846) and a low coefficient of variation (CV = 0.71%) further indicated the model's reliability and accuracy. Next, a climbing method was employed to optimize the experiment and identify a suitable solution using the mathematical equations within the model. The optimized results obtained through the climbing method are presented in Table 5.

The optimization process identified condition number 4 in Table 5 as the optimal solution, where the composite function (Y_{conv}) reached its maximum value (Y_{conv} = 301.312). This composite function represents a balance between the individual objective functions (Y1, Y2, and Y3). The results of the content of components Y1, Y2, and Y3 are somewhat different from previous

Table 5: Experimental data according to the climbing method of the linear convolution function (Y_{conv})

Number	enzyme	temperature	time	RDS	SDS	RS	Y_{conv}	
1	119.887	58.266	179.621	490.073	150.347	357.557	301.176	
2	119.609	57.772	178.381	490.176	152.143	355.712	301.177	
3	119.422	56.762	176.159	490.425	155.693	352.006	301.165	
4	***119.384***	***57.051***	***179.890***	***489.390***	***151.236***	***357.348***	***301.312***	***selected***
5	119.428	56.572	178.357	489.604	153.613	354.823	301.295	
6	119.375	57.605	177.933	490.351	152.558	355.148	301.153	
7	118.282	56.064	177.896	490.646	153.044	354.435	301.121	
8	119.529	55.194	176.281	490.132	157.431	350.596	301.237	
9	119.640	53.114	179.162	490.396	155.822	351.968	301.195	
10	118.782	56.238	179.752	489.681	151.551	356.788	301.272	

Table 6: Comparison of climbing experiments values with each objective function

Objective function	enzyme	temperature	time	RDS	SDS	RS	Y_{conv}
Y1	116.593	57.620	174.588	**490.886**	154.159	353.103	**301.082**
Y2	60.000	65.971	128.247	569.623	**243.550**	188.167	**268.611**
Y3	110.477	63.071	179.625	505.053	132.013	**361.613**	**298.461**
Y_{conv}	119.384	57.051	179.890	489.390	151.236	357.348	**301.312**

studies such as Le Thi Bich Phuong et al. [17], Doan Thu Huong et al. [22], and Zhang et al. [23]. However, this difference is due to the use of different raw materials and different enzyme sources.

To assess the effectiveness of the convolution function, the research compared the function to the individual objective functions (Table 6). The comparison revealed that the Y_{conv} of each objective function against Y_{conv} from the convolution function, we observe that the convolution function satisfies the condition ($Y_{convmax}$) = 301.312 > Y_{conv} values of each objective function.

V. CONCLUSION

To achieve the desired levels of RDS, SDS, and RS simultaneously, the optimal initial conditions for the saccharification process include glucoamylase concentration = 119.384 U/mL, temperature = 57.051^{o}C, and time = 179.890 minutes. These values were identified through the application of a linear convolution method combined with a climbing method for optimization. The convolution function satisfies the condition ($Y_{convmax}$), corresponding to objective functions Y1 = 489.390 mg/g, Y2 = 151.236 mg/g, and Y3 = 357.348 mg/g.

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